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botulism poisoning. These conclusions have been supported by studies conducted by Netter in France and by Simon Flexner in this country. Moreover, botulism is comparatively rare in the United States, whereas numerous cases of sleeping sickness have been recognized in countries where botulism is unknown and canned foods have not been consumed. Sleeping sickness is a febrile disease, associated with early symptoms of the respiratory tract and distinct inflammatory change in the brain tissue. These lesions are not found in botulism poisoning, and the temperature in this intoxication is usually subnormal. Single, rather than multiple, cases of sleeping sickness have been observed in family and other intimate groups of persons. On the other hand, botulism poisoning affects, as a rule, several members of the same household or institution, and the source of the poison can be traced, by the experienced investigator, to some food. Botulism can be prevented by thoroughly cooking all canned vegetables or fruits which have not been previously processed at high temperatures, after they are removed from the container.

DIRECT INOCULATION TEST FOR B. BOTULINUS TOXIN.

Determination of the Presence of B. Botulinus Toxin by Intraperitoneal Inoculation of Laboratory Animals with Suspected Foods.

By I. A. BENGTSON, Bacteriologist, Hygienic Laboratory, United States Public Health Service.

Attention is called to the rapidly fatal effects, with characteristic symptoms, of large doses of toxin and cultures of *Bacillus botulinus* when administered to small laboratory animals, particularly the mouse and guinea pig, by the intraperitoneal route. Results obtained in experiments carried out at the Hygienic Laboratory have suggested the procedure as a simple and expeditious test for the determination of the presence of botulinus toxin in suspected canned foods and, therefore, of use as an aid to the earlier diagnosis of botulism in case the food is available. By direct inoculation of white mice or guinea pigs with the food substance, the delay occasioned by filtration or by isolation of the organism, which may require several days, may be obviated.

The subcutaneous inoculation of guinea pigs or the feeding of the suspected food to chickens has been the method most frequently used in determining the presence of a toxin in suspected food. Graham and Schwarze (1) have recently shown that Type A strains of *B. botulinus* may be identified by feeding suspected contaminated food to chickens, which are not susceptible to the Type B strains. Symptoms appear in from 5 to 6 hours and death occurs after 18 to 24 hours.

The intraperitoneal inoculation of animals is also suggested in the examination of stomach contents for the detection of the presence of *B. botulinus* toxin, and it is possible that inoculation of mice with citrated blood from suspected botulism patients may give a clue to the presence of the toxin in the circulating blood. The method may be used also for the rapid identification and differentiation of the two types of *B. botulinus*, A and B, in experimental work.

That the mouse is highly susceptible to the toxin of *B. botulinus* was observed by von Ermengem, who found that as small a dose as 0.000025 c. c. of toxin was fatal to this animal when inoculated subcutaneously. That massive doses of toxin administered intraperitoneally have a rapidly fatal effect has been recently reported by Orr (2), who states that mice inoculated intraperitoneally died within a period of four hours.

Forssman (3) describes the symptom complex in rabbits and guinea pigs inoculated intraperitoneally or intrapleurally with toxin, as compared with that produced on subcutaneous, intracerebral, or subdural inoculation. In the case of the latter, respiratory symptoms are not pronounced until just before death. Following inoculation into the peritoneal cavity or into the pleura or the lungs, on the other hand, the most prominent symptom is dyspnea, the respiration frequency falling rapidly from 120–160 to 20–30 per minute. The respiration is distinctly costal, with a strong contraction at each breath. Death is ascribed to paralysis of the diaphragm, and Forssman designates this form of botulism as the diaphragmal type. Massive doses of toxin inoculated intraperitoneally or intrapleurally into guinea pigs caused death in 4 hours, whereas the same amount inoculated subcutaneously required 6 hours.

The symptoms as observed in mice in the work here reported are very characteristic and appear to be even more distinctive than in the guinea pig. Soon after inoculation, the animals present a sunken-in appearance at the flanks, which has been described as "wasp-waisted." A pronounced contraction is observable at each breath. In the early stages the number of respirations sometimes appears to increase, but as symptoms develop, there is a gradual decrease in the number of respirations per minute, with increased effort at each breath, until the animal finally succumbs.

EXPERIMENTAL.

Preliminary tests were made with glucose broth cultures of Type A and B strains of *B. botulinus*, with the results indicated in Tables I and II. The inoculations throughout were by the intraperitoneal route unless otherwise indicated.

TABLE I.—*Effect of glucose broth cultures (5 days old) of B. botulinus injected intraperitoneally into mice.*

Number of mouse.	Type of culture.	Amount of culture.	Amount of antitoxin.	Time of appearance of symptoms.	Time of death.
		c. c.	*c. c.*		
1	B Nevin strain	0.25		3 hours	4 hours.
2	A Memphis strain	.25		2 hours	2½ hours.
3	Do	.25		2 hours	4 hours.
4	A Boise strain	.25		2 hours	2½ hours.
5	B Nevin strain	0.25	[1]0.25		Survived.
6	A Memphis strain	.25	[2].25		Do.
7	Do	.25	.25		Do.
8	A Boise strain	.25	.25		Do.

[1] 0.25 c. c. of Type B antitoxin represents about 20 units of Type B antitoxin. (According to the Hygienic Laboratory standard, 1 unit protects against 1,000 minimal lethal doses of toxin.)

[2] 0.25 c. c. of Type A antitoxin represents about 25 units of Type A antitoxin.

TABLE II.—*Effect of glucose broth culture (6 days old) of B. botulinus injected intraperitoneally into mice.*

Number of mouse.	Type of culture.	Amount of culture.	Time of death.
		c. c.	
1	A Boise culture	0.1	2 hours.
2	Do	.01	4 hours.
3	Do	.001	8 hours.
4	Do	.0001	22 hours.

The size of the doses of toxin Type A represented by 0.25 c. c. in Table I may be determined approximately by comparison with Table II. The minimal lethal dose of the Boise culture was less than 0.0001 c. c., and, therefore, 0.25 c. c. represents at least 2,500 minimal lethal doses.

A test was made on guinea pigs to determine the relative effects of inoculating the same amounts of culture subcutaneously and intraperitoneally. Results were obtained much more promptly by intraperitoneal than subcutaneous inoculation.

TABLE III.—*Effect of glucose broth cultures of B. botulinus injected intraperitoneally and subcutaneously into guinea pigs.*

Number of guinea pig.	Culture inoculated.	Amount of culture.	Method of injection.	Time of appearance symptoms.	Time of death.
		c. c.			
1	Glucose broth culture (Type A Memphis strain 8 days old).	2.0	Intraperitoneally		1 hour, 40 minutes.
2	Do	2.0	Subcutaneously	Not before 5 hours.	7 hours.

A series of tests was then carried out with food, and cans of the following foods were inoculated with the Type A Memphis and Boise strains: String beans, peas, spinach, olives, corn, and beets. Inoculations were made by introducing a very small amount of a month-old culture by means of a Pasteur pipette through a hole in the top of the can, the opening then being closed by solder. Incubation was carried out at a temperature of 37° C.

Two of the cans thus incubated, namely, the peas and corn, became "swells" within from one to two days, showing considerable bulging at both top and bottom. The can of peas inoculated with the Memphis strain was opened on the seventh day and tests were carried out on mice and guinea pigs.

The odor from this can was exceedingly offensive and the peas were more or less disintegrated. Inoculations were made into mice and the results shown in Table IV were obtained.

TABLE IV.—*Effect of intraperitoneal inoculations of mice with cultures of B. botulinus grown in canned peas.*

Number of mouse.	Amount of culture.	Antitoxin administered.	Amount of antitoxin.	Time of appearance of symptoms.	Time of death.
	c. c.		*c. c.*	*Hours.*	
1......	1.5	....	....	....	10 or 15 minutes
2......	1.5	....	....	....	Do.
3......	1.0	....	....	....	Do.
4......	1.0	....	....	....	Do.
5......	.5	....	....	¾	1¼ hours.
6......	.5	....	....	¾	1½ hours.
7......	1.0	Polyvalent antitoxin......................	[1]0.5	1	2¼ hours.
8......	1.0	Type B antitoxin.........................	[2].5	....	About 15 minutes.

[1] 0.5 c. c. of the polyvalent antitoxin represents about 30 units of Type A antitoxin.
[2] 0.5 c. c. of Type B antitoxin represents about 40 units of Type B antitoxin.

The very early deaths (within from 10 to 15 minutes) of the mice receiving doses of 1.0 and 1.5 c. c. can hardly be ascribed to the effect of toxin alone. Even the mouse receiving polyvalent antitoxin (No. 7) succumbed, though after a longer period. Further tests were then made with smaller amounts of culture, the results of which are shown in Table V.

TABLE V.—*Effect of intraperitoneal inoculations of mice with cultures of B. botulinus grown in canned peas.*

Number of animal.	Amount of culture.	Antitoxin administered.	Amount of antitoxin.	Time of appearance of symptoms.	Time of death.
Mouse:	*C. c.*		*C. c.*		
1..........	0.5	....	....	30 minutes..........	1 hour 10 minutes.
2..........	.5	....	....	35 minutes..........	2 hours 35 minutes.
3..........	.1	....	....	55 minutes..........	1 hour 20 minutes.
4..........	.1	....	....	40 minutes..........	2 hours 10 minutes.
5..........	.01	....	....	1 hour 25 minutes...	2 hours.
6..........	.01	....	....	.....do..............	2 hours 15 minutes.
Controls:					
7..........	.5	Polyvalent antitoxin..	[1]0.5	....	Survived.
8..........	.1	.....do..................	.5	....	Do.
9..........	.01	.....do..................	.5	....	Do.
10..........	.5	Type B antitoxin.....	[2].5	....	30 minutes.
11..........	.1	.....do..................	.5	30 minutes..........	1 hour 45 minutes.
12..........	.01	.....do..................	.5	1 hour 50 minutes...	2 hours.
Guinea pig:					
1..........	[3]1	....	....	2 hours 20 minutes..	3 hours.
2..........	[3]1	Polyvalent antitoxin..	[4]1	2 hours 50 minutes..	16 hours.
3..........	[4]1	....	....	.....do..............	3 hours 45 minutes.
4..........	[4]1	Polyvalent antitoxin..	[4]1	....	Survived.

[1] 0.5 c. c. of the polyvalent antitoxin represents about 30 units of Type A antitoxin.
[2] 0.5 c. c. of Type B antitoxin represents about 40 units of Type B antitoxin.
[3] Intraperitoneally.
[4] Subcutaneously.

The results obtained with the guinea pigs indicate that the administration of the antitoxin by the subcutaneous route, when the culture had been administered intraperitoneally, was not effective in this particular instance in neutralizing the toxin, probably because of the slower absorption when administered by this route and the great potency of the toxin.

The almost immediate death of the mice inoculated with the larger amounts of culture as shown in Table IV (1.5 c. c. and 1 c. c.) prompted the testing of a filtrate of the culture to determine whether the same amounts of this would cause death in as short a time. The mouse inoculated with 1½ c. c. of the filtrate became prostrated within from 5 to 10 minutes and died within 30 minutes, whereas the mouse inoculated with 1 c. c. also developed symptoms very early and appeared to be in a dying condition within 15 minutes, but later revived and then developed more severe symptoms and died in 2 hours and 10 minutes.

It has been suggested that excessive amounts of ammonia, which is evolved in the decomposition of proteins, might have been responsible for the very early effects produced in the mice receiving large doses. The behavior exhibited by the last mouse described above would point to some other substance than the toxin as being responsible for the early prostration.

The remaining cans were opened on the eighth day (string beans, spinach, olives, beets), the ninth day (corn), and the tenth day (string beans), and 1 c. c. of the juice of each can was inoculated into mice. The mice inoculated with samples from the two cans of string beans showed definite symptoms in less than 8½ hours and died in less than 22½ hours. The mouse inoculated with the spinach juice died in less than 22½ hours, no symptoms being observed up to 8½ hours. The remaining two mice inoculated with the samples from the canned beets and olives survived. Control mice inoculated with 0.5 c. c. of antitoxin and the same amounts of the food substances survived.

It can not be stated with certainty that toxin developed in the cans of spinach, olives, beets, and string beans, since they exhibited no offensive odor, nor was there any swelling of the cans as evidence of growth. It is possible that the small amount of inoculum may have contained sufficient toxin to account for the development of symptoms in animals. This being the case, the results obtained with the string beans and spinach would, nevertheless, show the value of the tests for demonstrating small amounts of toxin.

Two mice were inoculated with 0.25 c. c. each of the juice of the canned corn on the tenth day, one being protected by 0.5 c. c. of polyvalent antitoxin. Symptoms developed in the unprotected mouse in 1 hour, followed by death in 2 hours and 20 minutes. A control mouse inoculated with 0.5 c. c. of normal horse serum instead of antitoxin died in 1¼ hours. The protected mouse survived.

A single experiment was carried out to determine whether toxin might be present in appreciable amounts in the circulating blood of guinea pigs which had been fed with cultures. Two guinea pigs of 250–400 grams weight were fed with cabbage on which was sprayed 3 c. c. of a 9-day-old glucose broth culture of the Memphis strain. The following morning one pig was found dead and the other showed severe symptoms. The latter pig was bled from the heart and the blood collected in sodium citrate. Mice were then inoculated intraperitoneally with the citrated blood and also with the serum from some of the whole blood which had been allowed to clot, with the results recorded in Table VI.

TABLE VI.—*Effect of intraperitoneal inoculations of mice with the blood from guinea pigs affected with botulism.*

Number of mouse.	Amount of blood.	Symptoms.	Death.
	c. c.		
1	0.0001		Survived.
2	.001		Do.
3	.01		Do.
4	.1	22½ hours	27½ hours.
5	.5	4 hours	10½ hours.
6	1.0	2½ hours	23½ hours.
7	[1].5	Before 3 days 21 hours	3 days 21½ hours.

[1] Serum.

Control mice inoculated with 1 c. c., 0.5 c. c., and 0.1 c. c. of citrated blood from a normal guinea pig survived.

It is questionable whether toxin would be present in sufficient amounts in the blood of persons suffering with botulism to be detected by this method, but it is nevertheless suggested as a possibility.

DISCUSSION AND SUMMARY.

The results of the experiments here presented indicate that the intraperitoneal inoculation of mice or guinea pigs with suspected foods is a useful method for the quick determination of the presence of the toxin of *B. botulinus* in foods and for determining the type of organism present. By the inoculation of a series of three mice, one with culture alone, one previously inoculated with Type A antitoxin, and another with Type B antitoxin, it may be possible to determine within a short time which type is the causative organism and therefore which type of antitoxin should be used for treatment. In order to approximate the correct dose of toxin, it is suggested that 3 series be thus inoculated; one with 1.0 c. c. of culture, one with 0.5 c. c., and one with 0.1 c. c. Mice seem to be more favorable than guinea pigs for carrying out the tests, for the reason that symptoms usually develop more rapidly in mice and are more characteristic.

The length of time required for symptoms to develop is doubtless dependent on the amount of toxin present. The results of the tests carried out with the canned peas show how very rapidly fatal effects are brought about in laboratory animals when a large amount of toxin is present. In practice, food containing such excessive amounts of toxin would not, perhaps, be submitted for examination, as it is improbable that food in the condition exhibited by this particular can would ever be used for human consumption. It is, however, reasonable to conjecture that symptoms and death of mice inoculated with such foods as have been implicated in the recent outbreaks would occur within a reasonably short period and in time to be of material assistance in the diagnosis of the disease and the determination of the type of organism present.

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AN EPIDEMIOLOGICAL STUDY OF THE 1920 EPIDEMIC OF INFLUENZA IN AN ISOLATED RURAL COMMUNITY.

By CHAS. ARMSTRONG, Assistant Surgeon and Epidemiologic Aide, United States Public Health Service, with the Ohio State Department of Health; and ROSS HOPKINS, Assistant Epidemiologist, Ohio State Department of Health.

At a conference of Public Health Service officers held in Washington, D. C., in February, 1920, the desirability of making a series of intensive epidemiological studies of influenza in rural communities was discussed, and it was decided that the epidemiologic aides in the various States should undertake such studies as opportunities might present. Kelleys Island was selected for making this study in Ohio, because of its exceptional isolation and because of the severity of the 1920 epidemic at this place.

The Community Where This Study Was Made.

Kelleys Island, a political subdivision of Erie County, Ohio, is located in Lake Erie, about 12 miles north of Sandusky, 5 miles from Lakeside, and about the same distance from Put-in-Bay. The